SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Cloning, expression and purification of mAb C3.1 scFv - DNA encoding the nucleic acid sequences of the variable heavy and variable light chains of the C3.1 IgG3 mAb was synthesised by GeneArt (Life Technologies Corporation). The scFv was constructed in the variable heavy-linker $(GGGGS)_3$-variable light chain orientation with 5' Nco1 and 3' Not1 restriction sites. The DNA fragment was ligated into the pIT2 vector that was linearized following digestion with Nco1 and Not1 restriction endonucleases. The pIT2 vector adds 3' 6xHis and c-myc tags to the C3.1 construct so that subsequently the expressed polypeptide will contain C-terminal 6xHis and c-myc tags. The C3.1 construct was transformed into non-suppressor *E. coli* HB2151 cells for protein expression.

Histidine-tagged scFv fragments were solubly expressed in *E. coli* HB2151 and purified by immobilized metal affinity chromatography (IMAC). An overnight culture (10 ml) grown at 37 °C in terrific broth (TB) containing 75 μg/ml carbenicillin and 1.0 % glucose was used to seed 1L of TB containing 75 μg/mL carbenicillin and 0.1% glucose, and grown at 37 °C to an A_{600} = 0.9. Protein expression was induced with 1 mM ITPG and the culture was grown for 16 h at 24 °C. The culture was spun at 10,000 RPM for 10 min and the supernatant was pooled and 0.22 μm filtered prior to IMAC purification using an Akta Purifier (GE Healthcare). Filtered supernatant was run over a prepacked His-Trap™ column (GE Healthcare) equilibrated with IMAC buffer A (10 mM HEPES, 500 mM NaCl, pH 8.0). C3.1 scFv antibodies were eluted using a step-wise gradient (from 10 to 100%) of IMAC buffer B (10 mM HEPES, 500 mM NaCl, 500 mM imidazole, pH 8.0). Samples were further purified by gel filtration (Superdex75™ 10/300 GL; GE Healthcare), using IMAC buffer A as running buffer, to isolate monomeric scFv fractions. Following verification by SDS-PAGE and Western blotting, fractions containing monomeric scFv were concentrated and buffer exchanged into either PBS at pH 7.4 or 10 mM sodium phosphate at pH 7.2, using 10,000 MWCO Amicon spin filters (Millipore). Concentrations were calculated following absorbance measurements at 280 nm using extinction coefficient data. Purified scFv aliquots were either lyophilized or stored at −20°C.

Surface plasmon resonance analysis of ligand binding to scFv - Equilibrium studies were performed at 25°C on the Reichert SR7000DC (Reichert Technologies) surface plasmon resonance (SPR) instrument. C3.1 scFv antibodies were covalently immobilized to a carboxymethyl dextran-coated sensor ship (Reichert Technologies). The carboxymethyl groups of dextran were activated with *N*-ethyl-*N'*(3-dimethylaminopropyl) carbodiamide (EDC) and *N*-hydroxysuccinimide (NHS), and scFv antibodies were attached at pH 5.5 in 10 mM sodium acetate buffer. Unreacted moieties were blocked with 1.0 M ethanolamine, pH 8.5. ScFv antibodies were immobilized at levels of ~ 5000 RU in the experimental flow cell. All measurements were conducted in HEPES-buffered saline which contained 0.1 M HEPES and 1.5 M NaCl at pH 7.4.

Equilibrium analysis was performed at a flow rate of 35 μl/min, with 2 min analyte injection and 5 min buffer flow. Concentrations of **1**, **2** and **3** measured ranged from 0.97-500 μM. All experiments were double referenced, and were repeated 1-2 times on a Biacore X100 (GE Healthcare) SPR instrument. SPR signals were processed using Scrubber software (version 2.0; BioLogic) with a plain carboxymethyl dextran surface subtracted as a reference.